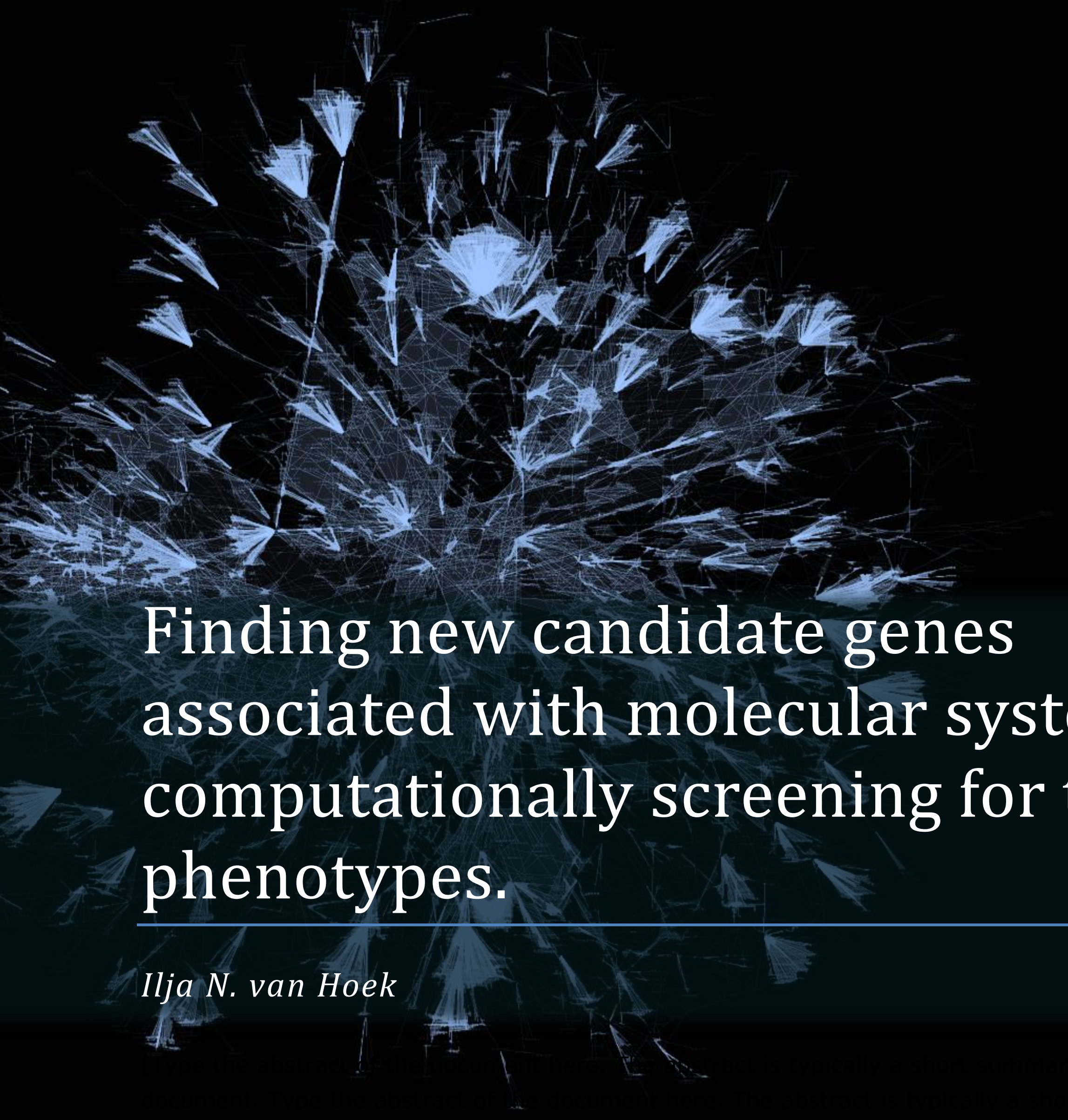

Finding new candidate genes associated with molecular systems by computationally screening for typical phenotypes.

Ilja N. van Hoek

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Abstract

The observance of phenotypes can be of important value in unraveling mechanisms of genetic disorders. Fortunately, human phenotypes linked to genetic defects are documented in online databases, one of which was built for facilitating computational analysis, the Human Phenotype Ontology (HPO) database. The observation of phenotypes on such a large scale has the potential of uncovering unknown molecular pathways. With mitochondrial disorders as research focus, mitochondrial proteins served as input in our new phenotype analysis tool, Galiphy. HPO phenotypes were scored based on their prevalence among the mitochondrial proteins prior to the calculation of the gene scores. All 3,246 genes in HPO were ranked on their scores after which statistical validation demonstrated that Galiphy could rank the genes according to their class, corresponding to areas under the ROC curves of ≥95%. Therefore, we propose to have successfully generated a 'phenotype fingerprint' using a simple but elegant scoring method. Specifically with this input, it revealed candidate genes which have a possible role in mitochondrial dysfunction. We conclude that ranking phenotypes using our bioinformatics tool can play a meaningful role in the set-up of experimental searches towards unravelling the molecular mechanisms behind genetic defects.

Table of Content

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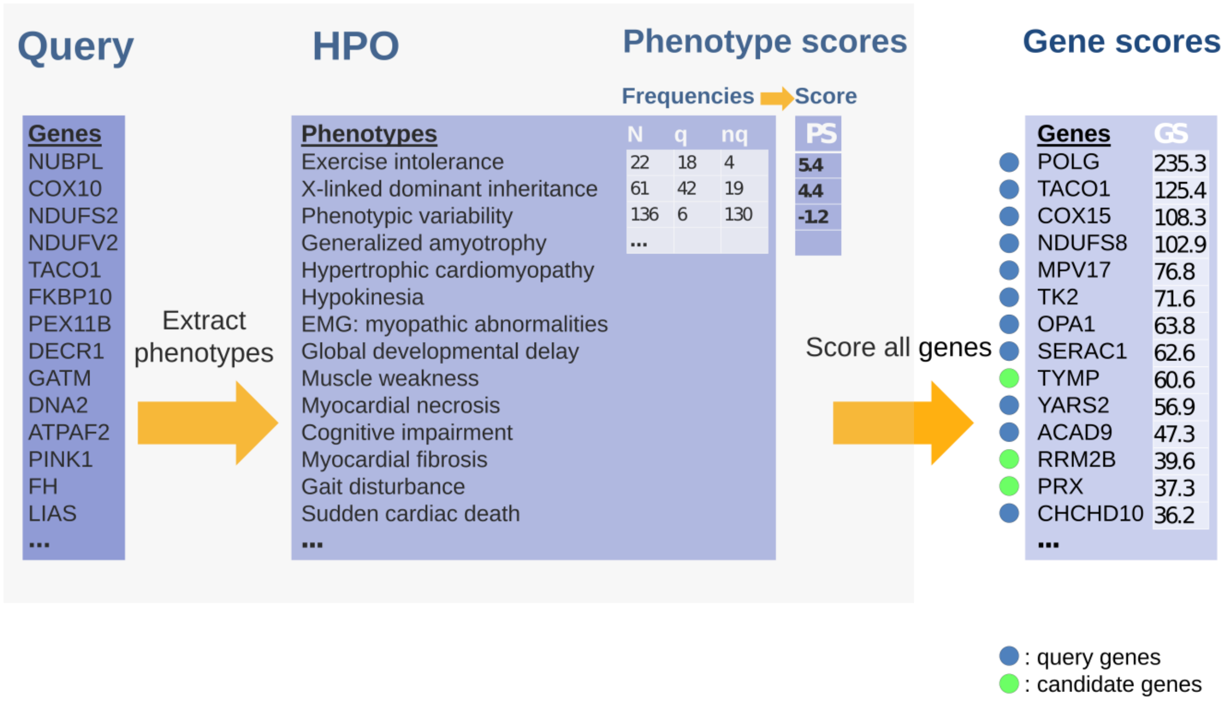
INTRODUCTION

Ever since mutations and phenotypes were considered linked, phenotypes have played a key role in genetic research. Among the first scientists to use this link were Beadle and Tatum in the 1940s[1] with their revolutionary pathway experiment. They deduced the arginine biosynthesis pathway from phenotype analysis of mutated *Neurospora.*

We used a similar deduction on a large-scale computational analysis, as we compiled a 'phenotype fingerprint' of a gene set by using phenotype data. In our approach, we used phenotype data from the Human Phenotype Ontology (HPO) database[2], which is the most comprehensive database with phenotypes linked to genes. HPO converted phenotypes from the Online Mendelian Inheritance in Man (OMIM) resource and the biomedical literature into a structured ontology of phenotype terms. This ontology includes over 11,000 phenotype terms, annotated to 3,246 disease genes. Our algorithm weighs the HPO phenotype terms according to their prevalence in a query gene set, as demonstrated in the grey box in Figure 1. Subsequently, all phenotypes are mapped back to their genes, scored and ranked by their associated weighted phenotypes (see right side of Figure 1). If the query genes share a functional relationship, the ranking can support the prediction of new genes.

In the study described in this report, we aim to shed light on the mechanisms of secondary mitochondrial disorders. The proteins affected in secondary mitochondrial disorders are not known to be associated with mitochondria, unlike primary mitochondrial disorders. Mitochondria are essential for many metabolic and cellular processes in the eukaryotic cell, including energy metabolism. Dysfunction of the mitochondria can result in disorders affecting the brain, skeletal muscle, heart, and liver. However, the clinical features are rarely pathognomonic[3], characterizing one of the main challenges encountered in diagnosing mitochondrial disorders (MD). This is because the exact same list of phenotypes can be caused by different mutations and,



Genes
NUBPL
COX10
NDUFS2
NDUFV2
TACO1
FKBP10
PEX11B
DECR1
GATM
DNA2
ATPAF2
PINK1
FH
LIAS
...

Phenotypes	N	q	nq	PS
Exercise intolerance	22	18	4	5.4
X-linked dominant inheritance	61	42	19	4.4
Phenotypic variability	136	6	130	-1.2
Generalized amyotrophy	...			
Hypertrophic cardiomyopathy				
Hypokinesia				
EMG: myopathic abnormalities				
Global developmental delay				
Muscle weakness				
Myocardial necrosis				
Cognitive impairment				
Myocardial fibrosis				
Gait disturbance				
Sudden cardiac death				
...				

	Genes	GS
🔵	POLG	235.3
🔵	TACO1	125.4
🔵	COX15	108.3
🔵	NDUFS8	102.9
🔵	MPV17	76.8
🔵	TK2	71.6
🔵	OPA1	63.8
🔵	SERAC1	62.6
🟢	TYMP	60.6
🔵	YARS2	56.9
🔵	ACAD9	47.3
🟢	RRM2B	39.6
🟢	PRX	37.3
🔵	CHCHD10	36.2
	...	

🔵 : query genes
🟢 : candidate genes

Figure 1. Schematic overview of Galiphy. The goal of the tool is to find new genes that have a similar phenotype as the query gene set. Here, mitochondrial proteins are used as query gene set. First, from a query gene set, HPO associates phenotypes to e.g. 80 of the 300 query genes, of which the phenotypes are extracted. Second, the abundance of each phenotype among the gene sets is determined ('q' are query genes, whereas 'nq' are non-query genes and 'N' are all genes in HPO) after which the phenotype score ('PS') can be calculated (see Equation 1). Lastly, for each gene present in HPO a gene score ('GS') can be determined by the combining of all phenotype scores (see Equation 2). Genes outside the initial query set are depicted with a green dot.

vice versa, same mutations can lead to different phenotypes.[4] For this reason, whole exome sequencing (WES) has recently been implemented as a state-of-the-art diagnostic test for MD by Wortmann *et al.*[4] In their study, histological, clinical, neuroradiological, metabolic, and biochemical data of 109 undiagnosed patients were thoroughly evaluated, after which they divided the patients into three groups based on level of suspicion of having MD. Subsequently, the WES analysis revealed 21 mutations in genes which were hitherto unknown to be localized to the mitochondrion, of which seven different genes were mutated in the patients with high suspicion of MD (SLC3A1/PREPL, NGLY1, ARID1B, SCN1A and three unpublished findings).

This leads us to our research question: how can a non-mitochondrial protein be responsible for mitochondrial dysfunction? Of which for instance two theories can be hypothesized. 1. Although Mitocarta[5] is the most comprehensive inventory of proteins localized in the mitochondrion, proteins absent from this list could be incorrectly designated as non-mitochondrial. 2. Alternatively, a non-mitochondrial protein could affect a pathway, which in turn influences mitochondrial function. In both cases, an unknown relationship with mitochondrial function is to be discovered. We therefore reasoned that Galiphy could help investigating the mechanisms of mitochondrial dysfunction by providing prioritization of genes based on mitochondrial typical phenotypes

MATERIAL & METHODS

Data sets

To collect phenotypes caused by disease genes, a phenotype database was required that contained appropriate annotation of phenotypes to genes. A controlled vocabulary of phenotypes structured in a manually curated systematic ontology was provided by the Human Phenotype Ontology (HPO) database[2]. They specifically developed a human phenotype database to facilitate comparison of phenotypes. HPO terms are organized in an ontology, in which terms are arranged in "is-a" relationships. This relationship is transitive, meaning that a genes that is annotated to a child term (specific HPO term) inherits all parent terms (more general HPO terms) up all paths to the root.

The file containing the *genes-to-phenotypes* annotations was downloaded (on 11/26/2015, version 5.1.73), restricting ourselves to the most specific HPO terms for the genes. This file links 5,881 different phenotype terms to 3,246 genes, which annotates 26 phenotype terms per gene on average.

In order to collect all human mitochondrial proteins for our query gene lists, the Human Mitocarta[5] version 2.0 was downloaded on 11/26/2015.

Score calculation procedure

As a query for the algorithm, a set of genes was established. Subsequently, we defined a formula for phenotypes based on the following principles: 1. Phenotypes that are more often associated to the query genes should score higher than phenotypes that are more often associated to the non-query genes. 2. If, for instance, HPO annotates 300 genes of the query, the remaining 3000 genes are automatically defined as non-query genes. 3. Prior is taken into account, meaning that the relative chances of finding phenotypes in each query list are incorporated. For each phenotype, we define its *Phenotype Score* (PS) as:

$$PS = log2 \frac{\frac{q|Q}{total\ Q}}{\frac{nq|nQ}{total\ nQ}}$$

Equation 1

where *q* is the abundance of the phenotype among the query gene list *Q*, and *nq* is the abundance of the phenotype among the non-query gene list *nQ*. Note, if a phenotype is associated with zero genes in a gene set, its frequency parameter (*n* or nq) is set to 0.1 to avoid dividing by zero. Once all 5881 phenotypes were given a score, the phenotypes were mapped back to their associated genes. For each gene, its phenotype set is defined by *P* = *{p*$_1$*, p*$_2$*,…,p*$_i$*},* and a *Gene Score* 'GS' was calculated by:

$$\mathrm{GS} = \sum_{p_i \in P} PS$$

Equation 2

This score represents to which extent the gene is associated to phenotypes typical for the query gene set. After all HPO genes were given a gene score, further analysis was performed to evaluate the performance of the algorithm.

Query gene lists

The first query gene list was compiled by selecting all Mitocarta genes that were annotated by HPO, remaining 351 genes. Analysis of the first output revealed that six of the 351 mitochondrial proteins caused a bias in the algorithm. The phenotypes of the succinate dehydrogenase genes were not congenital, these genes were therefore eliminated from the second query gene list. To make the scoring specific for MD phenotypes, the mitochondrial protein list was manually curated and 168 proteins directly responsible for energy conversion were selected for the third query gene list. The genes in the three different queries are listed in Appendix I.

Performance analysis of Galiphy

We conducted a gene set enrichment analysis (GSEA)[6] to test the robustness of the algorithm. The null hypothesis of GSEA is defined as follows: genes belonging to predefined classes are randomly distributed when ranked according to a specific parameter. Thus, by ranking all HPO genes by their gene score, the quality of the division between query genes and non-query genes was measured. In order to perform Bayesian analyses, we define the negative gene set as the non-mitochondrial proteins that were in the Mitocarta training set of Calvo *et al*[5], classified as 'non_mito'. Positive predictive value graphs and receiving operating characteristic curves were made for each query gene list to illustrate how the query genes are represented among the highest scoring genes.

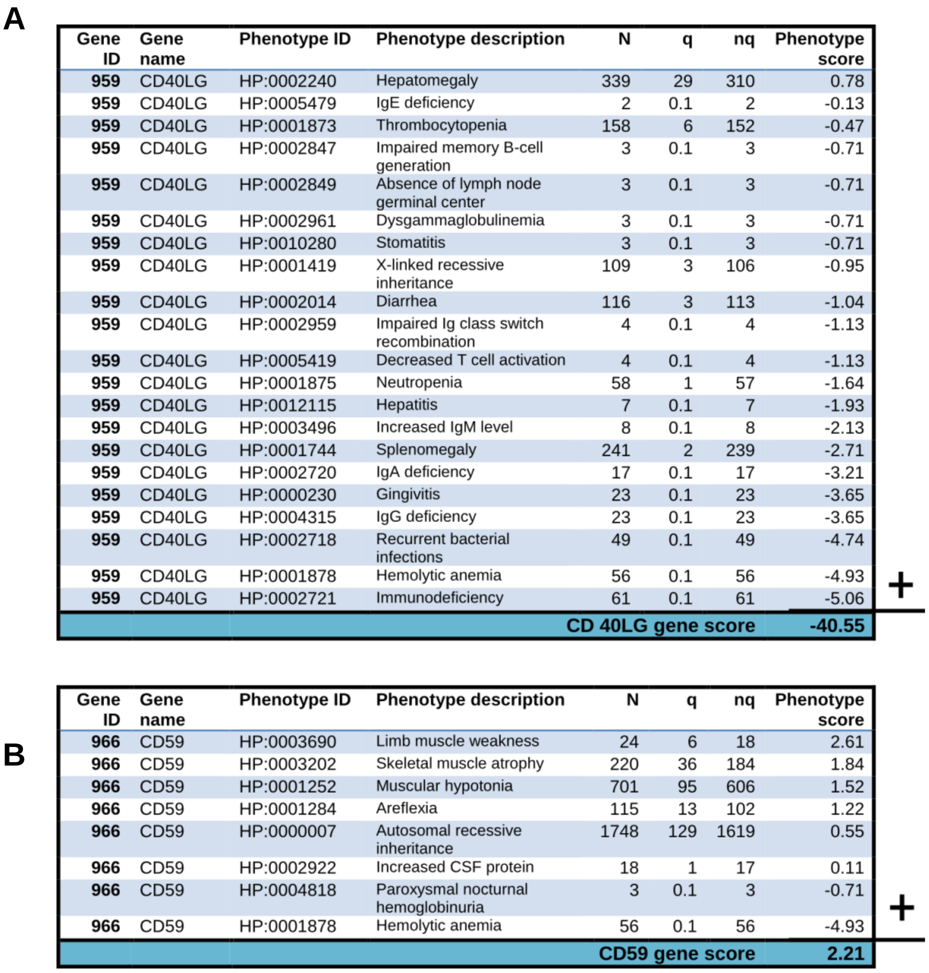
Further functional analyses were performed on the high scoring non-query genes (i.e., candidate genes) in order to gain insight in the possible relation with MD. String[7] version 10.0 was used for the top 200 candidate genes (on 6/14/2016). For genes that had individually caught our attention, we obtained co-expression information from the WeGET[8] tool (data obtained on 4/6/2016, version 1.0).

RESULTS & DISCUSSION

Using a query of mitochondrial genes, our algorithm generated an output of specificity scores for all phenotypes and genes in the HPO database. The query gene list consists of the genes linked to at least one phenotype in the HPO database. Phenotypes were scored according to their prevalence among query genes and non-query genes. These phenotype scores are representative for the level of particularity of the phenotype for the query genes. It is important to note that observation of the phenotype scores is not only relevant for fundamental biochemical questions, but also for clinical applications. The phenotype scores (PS) combined resulted in a gene score (GS) (see Equation 1 and 2 in *Material & Methods*). The establishment of the GS of two example genes are demonstrated in Figure 2. The scoring of a gene indicated how typical its phenotypes are for the query gene set. When the list of all genes were rank-ordered by GS, the high scoring non-query genes, i.e., candidate genes were further analyzed. In this study, the high scoring non-query genes were of particular interest and therefore studied in more detail. This could enlighten processes linked to the query genes. In addition, the candidate genes were further analyzed with the String enrichment tool.

Establishing adjusted query gene sets

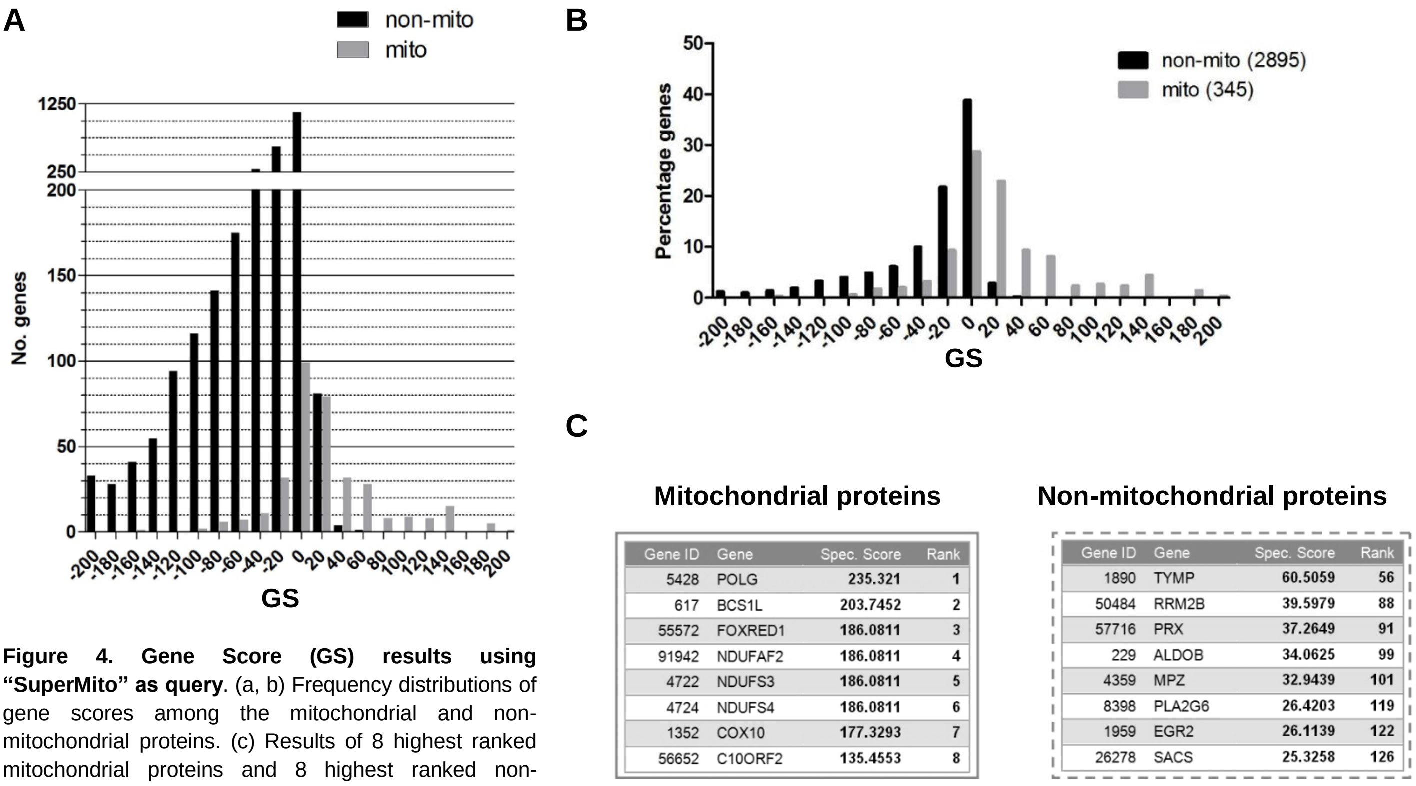
The first query generated an output of which the top 50 highest GS (ranging from 281.91 to 86.91) contained five complex IV genes, five complex II and 23 complex I genes. The top 50 highest scoring phenotypes were examined by a physician specialized in MD. Surprisingly, some of these phenotypes were assigned as not typical for MD. The succinate dehydrogenase genes caused the appearance of tumor-related phenotypes to score among the highest ranked phenotypes (as is illustrated in Appendix II). Although its mechanism is not fully understood, dysfunction of the complex II succinate dehydrogenase can lead to the formation of pheochromocytoma (PHEO) and paraganglioma (PGL)[9]. The genes caused bias in the algorithm, as genes that



Gene ID	Gene name	Phenotype ID	Phenotype description	N	q	nq	Phenotype score
959	CD40LG	HP:0002240	Hepatomegaly	339	29	310	0.78
959	CD40LG	HP:0005479	IgE deficiency	2	0.1	2	-0.13
959	CD40LG	HP:0001873	Thrombocytopenia	158	6	152	-0.47
959	CD40LG	HP:0002847	Impaired memory B-cell generation	3	0.1	3	-0.71
959	CD40LG	HP:0002849	Absence of lymph node germinal center	3	0.1	3	-0.71
959	CD40LG	HP:0002961	Dysgammaglobulinemia	3	0.1	3	-0.71
959	CD40LG	HP:0010280	Stomatitis	3	0.1	3	-0.71
959	CD40LG	HP:0001419	X-linked recessive inheritance	109	3	106	-0.95
959	CD40LG	HP:0002014	Diarrhea	116	3	113	-1.04
959	CD40LG	HP:0002959	Impaired Ig class switch recombination	4	0.1	4	-1.13
959	CD40LG	HP:0005419	Decreased T cell activation	4	0.1	4	-1.13
959	CD40LG	HP:0001875	Neutropenia	58	1	57	-1.64
959	CD40LG	HP:0012115	Hepatitis	7	0.1	7	-1.93
959	CD40LG	HP:0003496	Increased IgM level	8	0.1	8	-2.13
959	CD40LG	HP:0001744	Splenomegaly	241	2	239	-2.71
959	CD40LG	HP:0002720	IgA deficiency	17	0.1	17	-3.21
959	CD40LG	HP:0000230	Gingivitis	23	0.1	23	-3.65
959	CD40LG	HP:0004315	IgG deficiency	23	0.1	23	-3.65
959	CD40LG	HP:0002718	Recurrent bacterial infections	49	0.1	49	-4.74
959	CD40LG	HP:0001878	Hemolytic anemia	56	0.1	56	-4.93
959	CD40LG	HP:0002721	Immunodeficiency	61	0.1	61	-5.06
			CD 40LG gene score				-40.55

Gene ID	Gene name	Phenotype ID	Phenotype description	N	q	nq	Phenotype score
966	CD59	HP:0003690	Limb muscle weakness	24	6	18	2.61
966	CD59	HP:0003202	Skeletal muscle atrophy	220	36	184	1.84
966	CD59	HP:0001252	Muscular hypotonia	701	95	606	1.52
966	CD59	HP:0001284	Areflexia	115	13	102	1.22
966	CD59	HP:0000007	Autosomal recessive inheritance	1748	129	1619	0.55
966	CD59	HP:0002922	Increased CSF protein	18	1	17	0.11
966	CD59	HP:0004818	Paroxysmal nocturnal hemoglobinuria	3	0.1	3	-0.71
966	CD59	HP:0001878	Hemolytic anemia	56	0.1	56	-4.93
			CD59 gene score				2.21

Figure 2. Phenotype scoring and subsequent gene scoring of two example genes. HPO assigned 21 phenotypes to CD40LG. The phenotype "Hepatomegaly" is assigned to 339 (*N*) out of 3246 genes, of which 29 (*q*) out of 168 genes are query genes and 310 (*nq*) out of 3079 are non-query genes. Each phenotype is scored as shown in the last column. This phenotype score represents how specific the phenotype is for the query genes. A higher phenotype score indicates that the phenotype is 'typical' for the query genes. The phenotype scores of all 21 phenotypes linked to CD40LG are combined and the resulting gene score is shown in the last row. A higher gene score indicates that the gene has a phenotypically outlook which is similar to the query genes.



Mitochondrial proteins

Gene ID	Gene	Spec. Score	Rank
5428	POLG	235.321	1
617	BCS1L	203.7452	2
55572	FOXRED1	186.0811	3
91942	NDUFAF2	186.0811	4
4722	NDUFS3	186.0811	5
4724	NDUFS4	186.0811	6
1352	COX10	177.3293	7
56652	C10ORF2	135.4553	8

Non-mitochondrial proteins

Gene ID	Gene	Spec. Score	Rank
1890	TYMP	60.5059	56
50484	RRM2B	39.5979	88
57716	PRX	37.2649	91
229	ALDOB	34.0625	99
4359	MPZ	32.9439	101
8398	PLA2G6	26.4203	119
1959	EGR2	26.1139	122
26278	SACS	25.3258	126

Figure 4. Gene Score (GS) results using "SuperMito" as query. (a, b) Frequency distributions of gene scores among the mitochondrial and non-mitochondrial proteins. (c) Results of 8 highest ranked mitochondrial proteins and 8 highest ranked non-mitochondrial proteins.

cause cancerous phenotypes are not congenital defects. In order to keep the query a gene list with only congenital genes, scores were generated with a second query in which the six SDH genes were excluded. The output of this query "MitoSDHrem" list was similar to the first output: for example, among the top 50 highest GS (now ranging from 186.76 to 68.97) the same complex IV and complex I genes appeared.

The genetic defects leading to mitochondrial disorders were of main interest in this research. Therefore, we changed our focus on genes in which mutations cause MD. We hypothesized that restricting the input to only

168 genes responsible for oxidative phosphorylation function would result in an output which prioritizes more specific for mitochondrial disorders than the previous two queries. Using this "SuperMito" query indeed resulted in specific scoring phenotypes for congenital MD (Appendix III). The gene scores (Figure 3a and b) indicated how typical the gene is associated with the query genes' phenotypes. A distribution of the phenotype scores for the different query genes can be found in Appendix IV. For simplicity, the following analysis of the prioritized genes only describe the results from the "SuperMito" query.

Candidate genes

Interestingly, some of the candidate genes have previously been suggested to be associated with mitochondrial function (Figure 3c). Mutations in both TYMP and RRM2B are known to cause mitochondrial DNA depletion syndromes.[10] The PLA2G6 gene has been suggested to be involved in mitochondrial function.[11]

We used the top 200 highest scoring non-mitochondrial proteins (i.e., our candidate genes) for analysis with String[7], which placed the genes in a context of an association network. In addition, String provided us the enrichment of the genes among KEGG pathways and GO biological processes. The genes were highly represented in metabolic processes and pathways, as well as N-glycan biosynthesis, bile secretion and the lysosome pathway (Appendix III).

We sought additional support for linking the candidate genes to mitochondria by including co-expression information. We used a p-value quantification provided by the weighted gene co-expression tool WeGET[8], using the "SuperMito" list as a query.

Statistical evaluation

In addition to the manual analysis of the output, the performance of the algorithm was also evaluated statistically. To illustrate the ability of the scoring method to classify mitochondrial genes, all HPO genes were rank-ordered by their score and gene set enrichment analysis[6] (GSEA) was performed (Figure 4a and b).

In addition, receiver operating characteristic (ROC) analysis allowed evaluation of the predictive classification performance of the algorithm (Figure 4c).

Lastly, the precision of the algorithm was demonstrated by a precision rank plot (Figure 4d). No cross-validation was used as statistical measurement, as the scoring method did not allow setting a threshold.

Considering that the "MitoAll" and "MitoSDHrem" had only a difference of six genes so their overall similar performance was not surprising. Fortunately, the overall higher performance of the "SuperMito" gene set satisfied our expectations.

Selection of a gene for experimental validation

Twelve genes from the WES analysis list of patients with secondary mitochondrial disorders mentioned earlier were among the top 300 candidate genes. Also, three

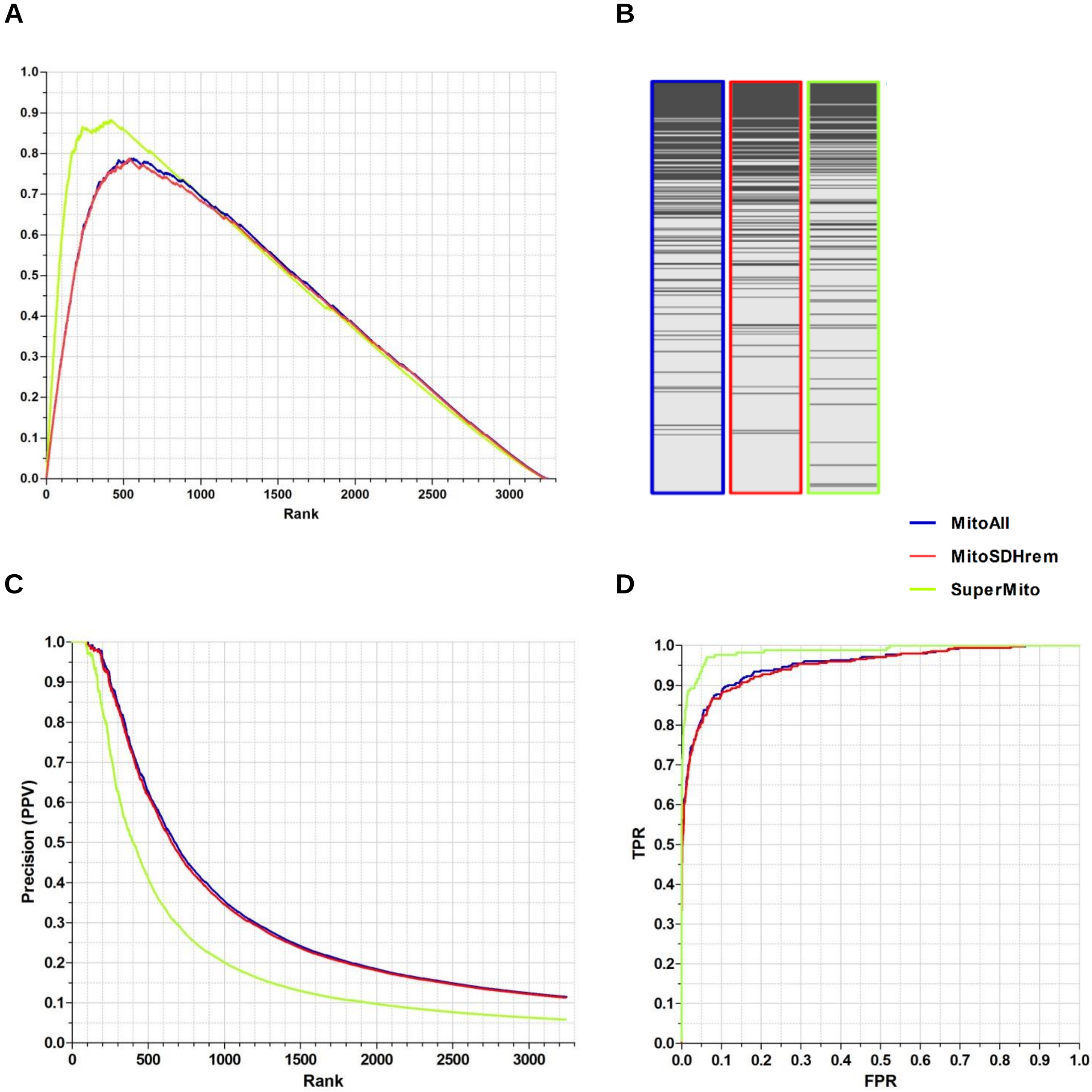


Figure 5. Statistical analyses depict that genes are ranked according to their mitochondrial phenotype. (a) Gene set enrichment plot shows that query genes are overrepresented among top-ranked genes, i.e. the leading edge set of genes before the peak of the graph. The peaks are found at rank 417, 535 and 564 for the SuperMito gene set (green), the MitoSDHrem gene set (red) and the MitoAll gene set (blue), respectively. (b) Schematic representation of disposition of the query genes in the rank-sorted list of all 3246 genes. (c) Precision rank plot shows that 50% of the genes are mitochondrial at rank 401, 653 and 674 for the gene sets SuperMito, MitoSDHrem and MitoAll, respectively. (d) Receiving operating characteristic graph tests the predictive ability of the scoring for the three query gene lists. AUC of the gene sets are 0.957, 0.949 and 0.952, for SuperMito, MitoSDHrem and MitoAll, respectively.

genes were significantly co-expressed with the "SuperMito" genes (Appendix VI). The NGLY1 gene and the EIF2B3 gene both belonged to these two groups. For NGLY1 mitochondrial association was suggested in literature[12].

Considerations of the tool

We introduced a new phenotype tool which is able to rank the genes associated to mitochondrial disorders according to their weighed phenotypes. Our results show that this type of scoring can be used in exploring gene functions. Moreover, Galiphy is also potentially useful in clinical applications. In essence, we only used simple computational methods, which is clearly an advantage as it can be exploited by every researcher or physician.

In the meantime, we will seek to improve our tool, which can be done in the following ways.

We encountered an issue regarding the ontology tree in the phenotype database. The HPO database assigns phenotypes to genes, but some phenotypes are not described at detailed level, because for example the heterogeneity of a genetic defect or general symptom of a disorder. In contrast, a specific phenotype could be caused by a thoroughly studied disorder, particular symptoms of the disorder or a regular genetic defect. As a result, difference in detail level of phenotype leads to problems in the scoring method. The phenotype 'Nausea and vomiting' illustrates this ontology issue: this phenotype is linked to 79 genes, its child term 'Nausea' and 'Vomiting' are linked to 11 and 106 genes, respectively. These inconsistent frequencies cause propensity in the phenotype scoring because the phenotypes are not correlated as one would expect. Further research could be done on this ontology, where it must be investigated whether the use of cumulative frequency up all paths to the root could result in a better scoring output.

Another suggestion for research is to find whether there are correlations when observing the phenotypes of each gene, and their ontology parent terms. So there might be some phenotypes overrepresented originating from the same parent term in the ontology tree. This way, it can be established whether genes have phenotypes annotated which share a more general phenotype. If possible and pertinent, the frequencies of the shared phenotype can be combined within the scoring of one gene.

Other tools that use phenotype information for exploration of gene function, include PHIVE[13], eXtasy[14], Phen-Gen[15] (side project of HPO), Phevor[16]. However, all these tools require genomic information about patients of a disorder, i.e. SNVs (eXtasy), exome variance (PHIVE) and sequence data (Phen-Gen and Phevor). Whereas Galiphy only requires a simple list of genes of interest.

Overall, Galiphy differs from other phenotype exploration tools, mainly due to its simplicity: a set of multiple genes is used as a query and phenotypic data is used for making a 'phenotype fingerprint'. The researcher or physician solely needs an interest in a set of related genes, for instance a common disorder or a common pathway.

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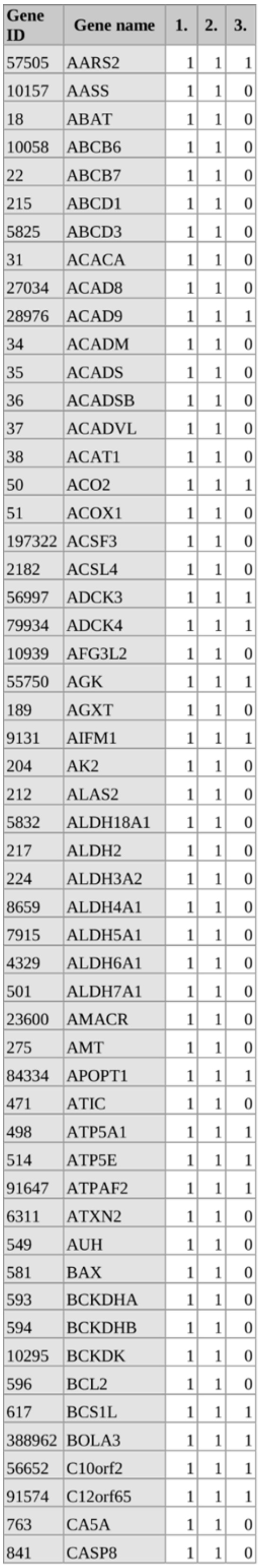
APPENDICES

Appendix I: All query gene lists used in this report.

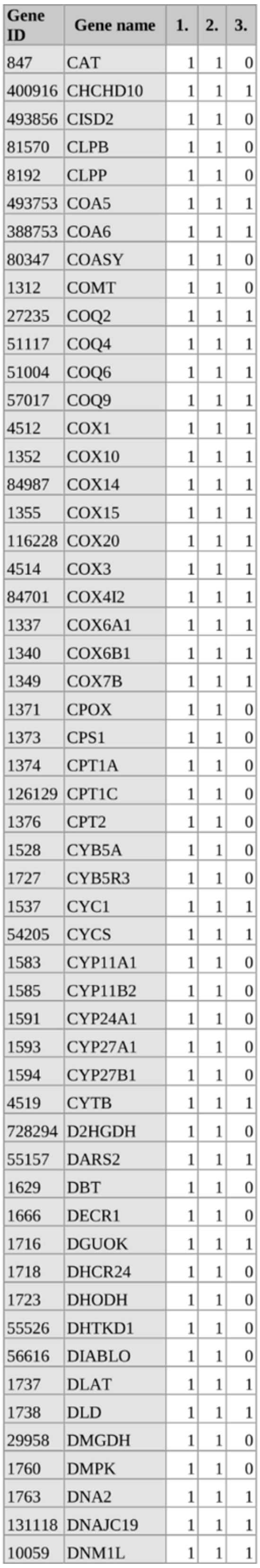
1: ‘MitoAll’. Of the 1158 genes from Mitocarta, 351 were associated to phenotypes by HPO.

2: ‘MitoSDHrem’. Same as list 1, but SDHx genes removed from list.

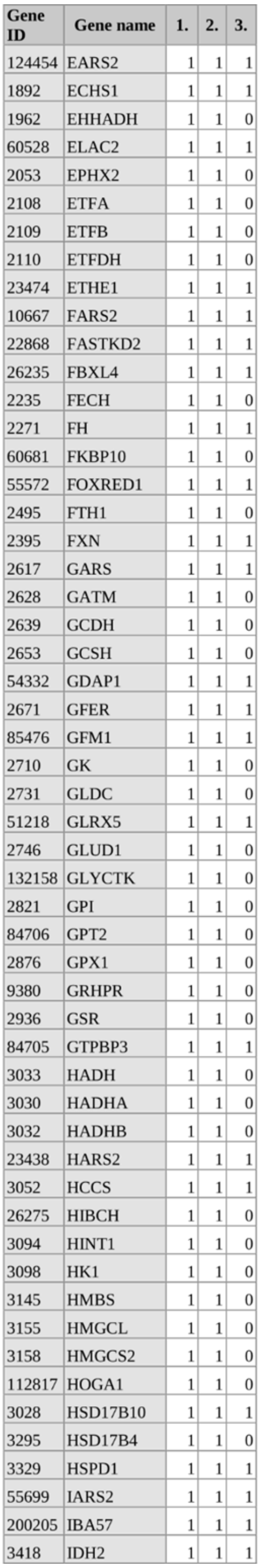
3: ‘SuperMito’. Selection of list 2, genes that are involved in energy production of mitochondrion.



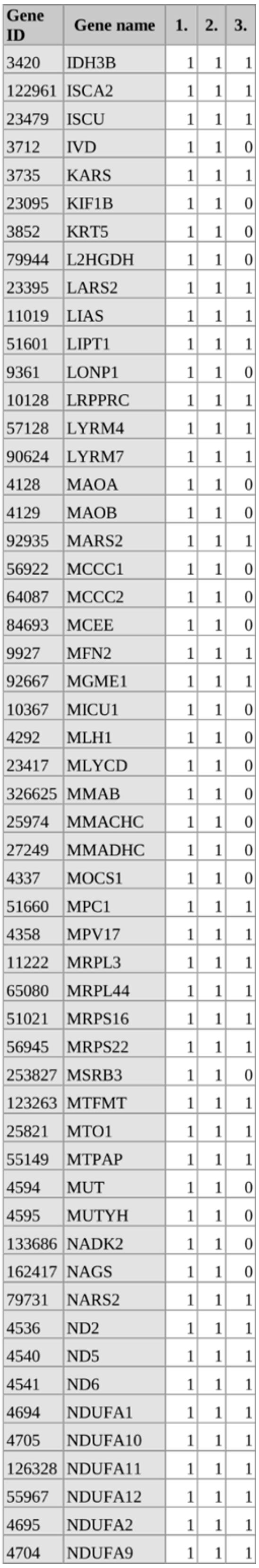
Gene ID	Gene name	1.	2.	3.
57505	AARS2	1	1	1
10157	AASS	1	1	0
18	ABAT	1	1	0
10058	ABCB6	1	1	0
22	ABCB7	1	1	0
215	ABCD1	1	1	0
5825	ABCD3	1	1	0
31	ACACA	1	1	0
27034	ACAD8	1	1	0
28976	ACAD9	1	1	1
34	ACADM	1	1	0
35	ACADS	1	1	0
36	ACADSB	1	1	0
37	ACADVL	1	1	0
38	ACAT1	1	1	0
50	ACO2	1	1	1
51	ACOX1	1	1	0
197322	ACSF3	1	1	0
2182	ACSL4	1	1	0
56997	ADCK3	1	1	1
79934	ADCK4	1	1	1
10939	AFG3L2	1	1	0
55750	AGK	1	1	1
189	AGXT	1	1	0
9131	AIFM1	1	1	1
204	AK2	1	1	0
212	ALAS2	1	1	0
5832	ALDH18A1	1	1	0
217	ALDH2	1	1	0
224	ALDH3A2	1	1	0
8659	ALDH4A1	1	1	0
7915	ALDH5A1	1	1	0
4329	ALDH6A1	1	1	0
501	ALDH7A1	1	1	0
23600	AMACR	1	1	0
275	AMT	1	1	0
84334	APOPT1	1	1	1
471	ATIC	1	1	0
498	ATP5A1	1	1	1
514	ATP5E	1	1	1
91647	ATPAF2	1	1	1
6311	ATXN2	1	1	0
549	AUH	1	1	0
581	BAX	1	1	0
593	BCKDHA	1	1	0
594	BCKDHB	1	1	0
10295	BCKDK	1	1	0
596	BCL2	1	1	0
617	BCS1L	1	1	1
388962	BOLA3	1	1	1
56652	C10orf2	1	1	1
91574	C12orf65	1	1	1
763	CA5A	1	1	0
841	CASP8	1	1	0



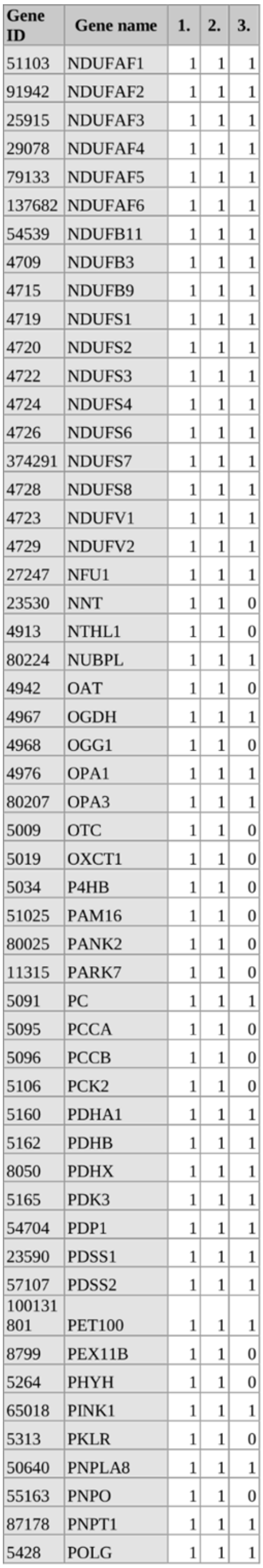
Gene ID	Gene name	1.	2.	3.
847	CAT	1	1	0
400916	CHCHD10	1	1	1
493856	CISD2	1	1	0
81570	CLPB	1	1	0
8192	CLPP	1	1	0
493753	COA5	1	1	1
388753	COA6	1	1	1
80347	COASY	1	1	0
1312	COMT	1	1	0
27235	COQ2	1	1	1
51117	COQ4	1	1	1
51004	COQ6	1	1	1
57017	COQ9	1	1	1
4512	COX1	1	1	1
1352	COX10	1	1	1
84987	COX14	1	1	1
1355	COX15	1	1	1
116228	COX20	1	1	1
4514	COX3	1	1	1
84701	COX4I2	1	1	1
1337	COX6A1	1	1	1
1340	COX6B1	1	1	1
1349	COX7B	1	1	1
1371	CPOX	1	1	0
1373	CPS1	1	1	0
1374	CPT1A	1	1	0
126129	CPT1C	1	1	0
1376	CPT2	1	1	0
1528	CYB5A	1	1	0
1727	CYB5R3	1	1	0
1537	CYC1	1	1	1
54205	CYCS	1	1	1
1583	CYP11A1	1	1	0
1585	CYP11B2	1	1	0
1591	CYP24A1	1	1	0
1593	CYP27A1	1	1	0
1594	CYP27B1	1	1	0
4519	CYTB	1	1	1
728294	D2HGDH	1	1	0
55157	DARS2	1	1	1
1629	DBT	1	1	0
1666	DECR1	1	1	0
1716	DGUOK	1	1	1
1718	DHCR24	1	1	0
1723	DHODH	1	1	0
55526	DHTKD1	1	1	0
56616	DIABLO	1	1	0
1737	DLAT	1	1	1
1738	DLD	1	1	1
29958	DMGDH	1	1	0
1760	DMPK	1	1	0
1763	DNA2	1	1	1
131118	DNAJC19	1	1	1
10059	DNM1L	1	1	1



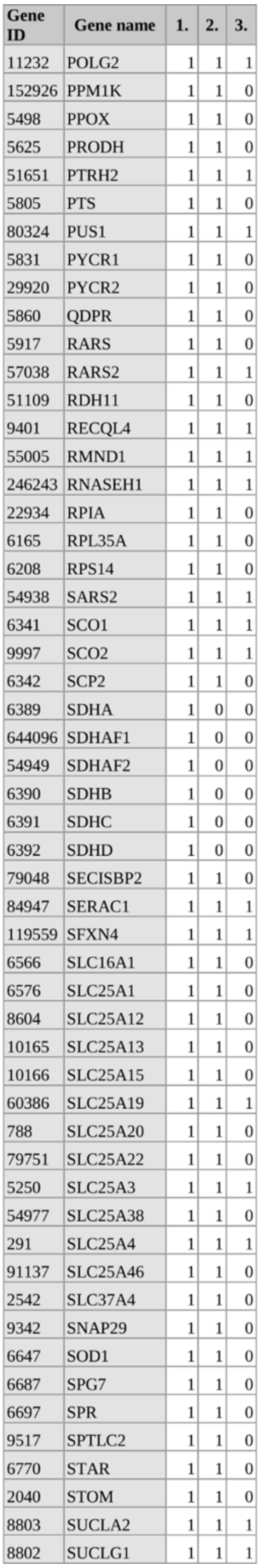
Gene ID	Gene name	1.	2.	3.
124454	EARS2	1	1	1
1892	ECHS1	1	1	1
1962	EHHADH	1	1	0
60528	ELAC2	1	1	1
2053	EPHX2	1	1	0
2108	ETFA	1	1	0
2109	ETFB	1	1	0
2110	ETFDH	1	1	0
23474	ETHE1	1	1	1
10667	FARS2	1	1	1
22868	FASTKD2	1	1	1
26235	FBXL4	1	1	1
2235	FECH	1	1	0
2271	FH	1	1	1
60681	FKBP10	1	1	0
55572	FOXRED1	1	1	1
2495	FTH1	1	1	0
2395	FXN	1	1	1
2617	GARS	1	1	1
2628	GATM	1	1	0
2639	GCDH	1	1	0
2653	GCSH	1	1	0
54332	GDAP1	1	1	1
2671	GFER	1	1	1
85476	GFM1	1	1	1
2710	GK	1	1	0
2731	GLDC	1	1	0
51218	GLRX5	1	1	1
2746	GLUD1	1	1	0
132158	GLYCTK	1	1	0
2821	GPI	1	1	0
84706	GPT2	1	1	0
2876	GPX1	1	1	0
9380	GRHPR	1	1	0
2936	GSR	1	1	0
84705	GTPBP3	1	1	1
3033	HADH	1	1	0
3030	HADHA	1	1	0
3032	HADHB	1	1	0
23438	HARS2	1	1	1
3052	HCCS	1	1	1
26275	HIBCH	1	1	0
3094	HINT1	1	1	0
3098	HK1	1	1	0
3145	HMBS	1	1	0
3155	HMGCL	1	1	0
3158	HMGCS2	1	1	0
112817	HOGA1	1	1	0
3028	HSD17B10	1	1	1
3295	HSD17B4	1	1	0
3329	HSPD1	1	1	1
55699	IARS2	1	1	1
200205	IBA57	1	1	1
3418	IDH2	1	1	1



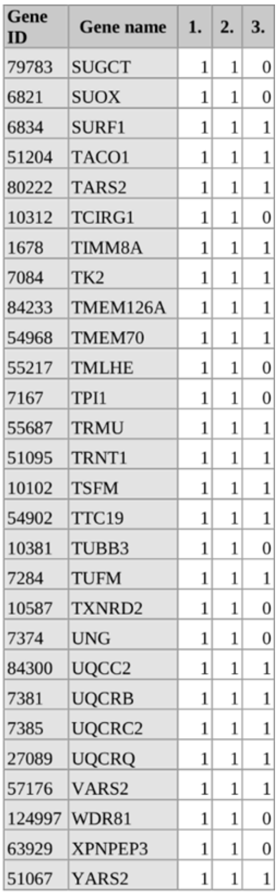
Gene ID	Gene name	1.	2.	3.
3420	IDH3B	1	1	1
122961	ISCA2	1	1	1
23479	ISCU	1	1	1
3712	IVD	1	1	0
3735	KARS	1	1	1
23095	KIF1B	1	1	0
3852	KRT5	1	1	0
79944	L2HGDH	1	1	0
23395	LARS2	1	1	1
11019	LIAS	1	1	1
51601	LIPT1	1	1	1
9361	LONP1	1	1	0
10128	LRPPRC	1	1	1
57128	LYRM4	1	1	1
90624	LYRM7	1	1	1
4128	MAOA	1	1	0
4129	MAOB	1	1	0
92935	MARS2	1	1	1
56922	MCCC1	1	1	0
64087	MCCC2	1	1	0
84693	MCEE	1	1	0
9927	MFN2	1	1	1
92667	MGME1	1	1	1
10367	MICU1	1	1	0
4292	MLH1	1	1	0
23417	MLYCD	1	1	0
326625	MMAB	1	1	0
25974	MMACHC	1	1	0
27249	MMADHC	1	1	0
4337	MOCS1	1	1	0
51660	MPC1	1	1	1
4358	MPV17	1	1	1
11222	MRPL3	1	1	1
65080	MRPL44	1	1	1
51021	MRPS16	1	1	1
56945	MRPS22	1	1	1
253827	MSRB3	1	1	0
123263	MTFMT	1	1	1
25821	MTO1	1	1	1
55149	MTPAP	1	1	1
4594	MUT	1	1	0
4595	MUTYH	1	1	0
133686	NADK2	1	1	0
162417	NAGS	1	1	0
79731	NARS2	1	1	1
4536	ND2	1	1	1
4540	ND5	1	1	1
4541	ND6	1	1	1
4694	NDUFA1	1	1	1
4705	NDUFA10	1	1	1
126328	NDUFA11	1	1	1
55967	NDUFA12	1	1	1
4695	NDUFA2	1	1	1
4704	NDUFA9	1	1	1



Gene ID	Gene name	1.	2.	3.
51103	NDUFAF1	1	1	1
91942	NDUFAF2	1	1	1
25915	NDUFAF3	1	1	1
29078	NDUFAF4	1	1	1
79133	NDUFAF5	1	1	1
137682	NDUFAF6	1	1	1
54539	NDUFB11	1	1	1
4709	NDUFB3	1	1	1
4715	NDUFB9	1	1	1
4719	NDUFS1	1	1	1
4720	NDUFS2	1	1	1
4722	NDUFS3	1	1	1
4724	NDUFS4	1	1	1
4726	NDUFS6	1	1	1
374291	NDUFS7	1	1	1
4728	NDUFS8	1	1	1
4723	NDUFV1	1	1	1
4729	NDUFV2	1	1	1
27247	NFU1	1	1	1
23530	NNT	1	1	0
4913	NTHL1	1	1	0
80224	NUBPL	1	1	1
4942	OAT	1	1	0
4967	OGDH	1	1	1
4968	OGG1	1	1	0
4976	OPA1	1	1	1
80207	OPA3	1	1	1
5009	OTC	1	1	0
5019	OXCT1	1	1	0
5034	P4HB	1	1	0
51025	PAM16	1	1	0
80025	PANK2	1	1	0
11315	PARK7	1	1	0
5091	PC	1	1	1
5095	PCCA	1	1	0
5096	PCCB	1	1	0
5106	PCK2	1	1	0
5160	PDHA1	1	1	1
5162	PDHB	1	1	1
8050	PDHX	1	1	1
5165	PDK3	1	1	1
54704	PDP1	1	1	1
23590	PDSS1	1	1	1
57107	PDSS2	1	1	1
100131801	PET100	1	1	1
8799	PEX11B	1	1	0
5264	PHYH	1	1	0
65018	PINK1	1	1	1
5313	PKLR	1	1	0
50640	PNPLA8	1	1	1
55163	PNPO	1	1	0
87178	PNPT1	1	1	1
5428	POLG	1	1	1



Gene ID	Gene name	1.	2.	3.
11232	POLG2	1	1	1
152926	PPM1K	1	1	0
5498	PPOX	1	1	0
5625	PRODH	1	1	0
51651	PTRH2	1	1	1
5805	PTS	1	1	0
80324	PUS1	1	1	1
5831	PYCR1	1	1	0
29920	PYCR2	1	1	0
5860	QDPR	1	1	0
5917	RARS	1	1	0
57038	RARS2	1	1	1
51109	RDH11	1	1	0
9401	RECQL4	1	1	1
55005	RMND1	1	1	1
246243	RNASEH1	1	1	1
22934	RPIA	1	1	0
6165	RPL35A	1	1	0
6208	RPS14	1	1	0
54938	SARS2	1	1	1
6341	SCO1	1	1	1
9997	SCO2	1	1	1
6342	SCP2	1	1	0
6389	SDHA	1	0	0
644096	SDHAF1	1	0	0
54949	SDHAF2	1	0	0
6390	SDHB	1	0	0
6391	SDHC	1	0	0
6392	SDHD	1	0	0
79048	SECISBP2	1	1	0
84947	SERAC1	1	1	1
119559	SFXN4	1	1	1
6566	SLC16A1	1	1	0
6576	SLC25A1	1	1	0
8604	SLC25A12	1	1	0
10165	SLC25A13	1	1	0
10166	SLC25A15	1	1	0
60386	SLC25A19	1	1	1
788	SLC25A20	1	1	0
79751	SLC25A22	1	1	0
5250	SLC25A3	1	1	1
54977	SLC25A38	1	1	0
291	SLC25A4	1	1	1
91137	SLC25A46	1	1	0
2542	SLC37A4	1	1	0
9342	SNAP29	1	1	0
6647	SOD1	1	1	0
6687	SPG7	1	1	0
6697	SPR	1	1	0
9517	SPTLC2	1	1	0
6770	STAR	1	1	0
2040	STOM	1	1	0
8803	SUCLA2	1	1	1
8802	SUCLG1	1	1	1



Gene ID	Gene name	1.	2.	3.
79783	SUGCT	1	1	0
6821	SUOX	1	1	0
6834	SURF1	1	1	1
51204	TACO1	1	1	1
80222	TARS2	1	1	1
10312	TCIRG1	1	1	0
1678	TIMM8A	1	1	1
7084	TK2	1	1	1
84233	TMEM126A	1	1	1
54968	TMEM70	1	1	1
55217	TMLHE	1	1	0
7167	TPI1	1	1	0
55687	TRMU	1	1	1
51095	TRNT1	1	1	1
10102	TSFM	1	1	1
54902	TTC19	1	1	1
10381	TUBB3	1	1	0
7284	TUFM	1	1	1
10587	TXNRD2	1	1	0
7374	UNG	1	1	0
84300	UQCC2	1	1	1
7381	UQCRB	1	1	1
7385	UQCRC2	1	1	1
27089	UQCRQ	1	1	1
57176	VARS2	1	1	1
124997	WDR81	1	1	0
63929	XPNPEP3	1	1	0
51067	YARS2	1	1	1

rank	HPO ID	Phenotype description	N	q	nq	PS
1	HP:0002490	Increased CSF lactate	49	49	0.1	11.98
2	HP:0008316	Abnormal mitochondria in muscle tissue	21	21	0.1	10.76
3	HP:0001404	Hepatocellular necrosis	18	18	0.1	10.54
4	HP:0006965	Acute necrotizing encephalopathy	18	18	0.1	10.54
5	HP:0012240	Increased intramyocellular lipid droplets	15	15	0.1	10.27
6	HP:0003348	Hyperalaninemia	10	10	0.1	9.69
7	HP:0006565	Increased hepatocellular lipid droplets	10	10	0.1	9.69
8	HP:0001414	Microvesicular hepatic steatosis	8	8	0.1	9.37
9	HP:0003150	Glutaric aciduria	8	8	0.1	9.37
10	HP:0001112	Leber optic atrophy	6	6	0.1	8.95
11	HP:0001117	Sudden loss of visual acuity	6	6	0.1	8.95
12	HP:0001129	Large central visual field defect	6	6	0.1	8.95
13	HP:0003219	Ethylmalonic aciduria	6	6	0.1	8.95
14	HP:0008972	Decreased activity of mitochondrial respiratory chain	6	6	0.1	8.95
15	HP:0001427	Mitochondrial inheritance	45	44	1	8.50
16	**HP:0000361**	**Pulsatile tinnitus (tympanic paraganglioma)**	**4**	**4**	**0.1**	**8.37**
17	**HP:0002377**	**Paraganglioma-related cranial nerve palsy**	**4**	**4**	**0.1**	**8.37**
18	**HP:0003001**	**Glomus jugular tumor**	**4**	**4**	**0.1**	**8.37**
19	**HP:0030074**	**Chemodectoma**	**4**	**4**	**0.1**	**8.37**
20	HP:0003530	Glutaric acidemia	4	4	0.1	8.37
21	HP:0006980	Progressive leukoencephalopathy	4	4	0.1	8.37
22	HP:0008314	Decreased activity of mitochondrial complex II	4	4	0.1	8.37
23	HP:0008344	Elevated plasma branched chain amino acids	4	4	0.1	8.37
24	**HP:0000740**	**Anxiety (with pheochromocytoma)**	**3**	**3**	**0.1**	**7.95**
25	**HP:0001011**	**Diaphoresis (with pheochromocytoma)**	**3**	**3**	**0.1**	**7.95**
26	**HP:0001606**	**Vocal cord paralysis (caused by tumor impingement)**	**3**	**3**	**0.1**	**7.95**
27	**HP:0001613**	**Hoarse voice (caused by tumor impingement)**	**3**	**3**	**0.1**	**7.95**
28	**HP:0001673**	**Tachycardia (with pheochromocytoma)**	**3**	**3**	**0.1**	**7.95**
29	**HP:0001676**	**Palpitations (with pheochromocytoma)**	**3**	**3**	**0.1**	**7.95**
30	**HP:0001686**	**Loss of voice**	**3**	**3**	**0.1**	**7.95**
31	**HP:0002331**	**Headache (with pheochromocytoma)**	**3**	**3**	**0.1**	**7.95**
32	**HP:0002640**	**Hypertension associated with pheochromocytoma**	**3**	**3**	**0.1**	**7.95**
33	**HP:0004897**	**Stress/infection-induced lactic acidosis**	**3**	**3**	**0.1**	**7.95**
34	**HP:0006737**	**Extraadrenal pheochromocytoma**	**3**	**3**	**0.1**	**7.95**
35	**HP:0006748**	**Adrenal pheochromocytoma**	**3**	**3**	**0.1**	**7.95**
36	HP:0002161	Hyperlysinemia	3	3	0.1	7.95
37	HP:0002614	Hepatic periportal necrosis	3	3	0.1	7.95
38	HP:0002686	Prenatal maternal abnormality	3	3	0.1	7.95
39	HP:0002928	Decreased activity of the pyruvate dehydrogenase (PDH) complex	3	3	0.1	7.95
40	HP:0003287	Abnormality of mitochondrial metabolism	3	3	0.1	7.95
41	HP:0003353	Propionyl-CoA carboxylase deficiency	3	3	0.1	7.95
42	HP:0003490	Defective dehydrogenation of isovaleryl CoA and butyryl CoA	3	3	0.1	7.95
43	HP:0003647	Electron transfer flavoprotein-ubiquinone oxidoreductase defect	3	3	0.1	7.95
44	HP:0006799	Basal ganglia cysts	3	3	0.1	7.95
45	HP:0011923	Decreased activity of mitochondrial complex I	3	3	0.1	7.95
46	HP:0011924	Decreased activity of mitochondrial complex III	3	3	0.1	7.95
47	**HP:0002886**	**Vagal paraganglioma**	**2**	**2**	**0.1**	**7.37**
48	**HP:0003334**	**Elevated circulating catecholamine level**	**2**	**2**	**0.1**	**7.37**
49	**HP:0006715**	**Glomus tympanicum paraganglioma**	**2**	**2**	**0.1**	**7.37**
50	HP:0001958	Nonketotic hypoglycemia	2	2	0.1	7.37
51	HP:0003344	3-Methylglutaric aciduria	2	2	0.1	7.37
52	HP:0003359	Decreased urinary sulfate	2	2	0.1	7.37
53	HP:0003489	Acute episodes of neuropathic symptoms	2	2	0.1	7.37
54	HP:0003572	Low plasma citrulline	2	2	0.1	7.37
55	HP:0003643	Sulfite oxidase deficiency	2	2	0.1	7.37

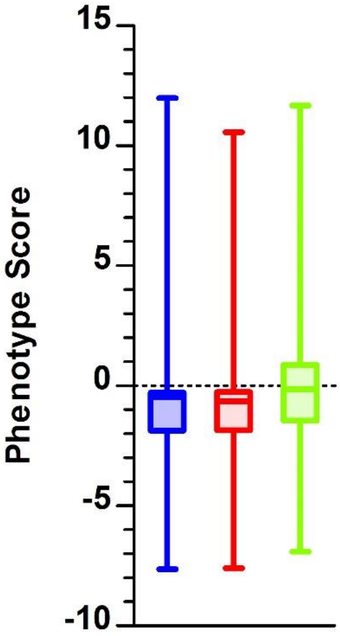
56	HP:0004448	Fulminant hepatic failure	2	2	0.1	7.37
57	HP:0004925	Chronic lactic acidosis	2	2	0.1	7.37
58	HP:0005974	Episodic ketoacidosis	2	2	0.1	7.37

Appendix II. Best scoring phenotypes with their frequencies (that is N, q and nq) sorted on phenotype scores (PS). The phenotypes in grey rows are solely annotated by succinate dehydrogenase genes, some of these phenotypes are not known to be a regular mitochondrial phenotype at all, but most likely originated from the pheochromocytoma / paraganglioma annotation of SDH-genes.

Appendix III Best scoring phenotypes of the SuperMito query. The light grey phenotypes ranked as 20-76 are not shown as they are all linked to solely one query gene, which results in a score of 7.52.

Rank	HPO ID	Phenotype description	N	q	nq	PS
1	HP:0006965	Acute necrotizing encephalopathy	18	18	0.1	11.69
2	HP:0006565	Increased hepatocellular lipid droplets	10	10	0.1	10.84
3	HP:0001414	Microvesicular hepatic steatosis	8	8	0.1	10.52
4	HP:0008972	Decreased activity of mitochondrial respiratory chain	6	6	0.1	10.10
5	HP:0001129	Large central visual field defect	6	6	0.1	10.10
6	HP:0001117	Sudden loss of visual acuity	6	6	0.1	10.10
7	HP:0001112	Leber optic atrophy	6	6	0.1	10.10
8	HP:0002490	Increased CSF lactate	49	48	1	9.78
9	HP:0002928	Decreased activity of the pyruvate dehydrogenase (PDH) complex	3	3	0.1	9.10
10	HP:0011924	Decreased activity of mitochondrial complex III	3	3	0.1	9.10
11	HP:0011923	Decreased activity of mitochondrial complex I	3	3	0.1	9.10
12	HP:0001427	Mitochondrial inheritance	45	43	2	8.62
13	HP:0200125	Mitochondrial respiratory chain defects	2	2	0.1	8.52
14	HP:0008443	Spinal deformities	2	2	0.1	8.52
15	HP:0008945	Loss of ability to w alk in early childhood	2	2	0.1	8.52
16	HP:0008347	Decreased activity of mitochondrial complex IV	2	2	0.1	8.52
17	HP:0004925	Chronic lactic acidosis	2	2	0.1	8.52
18	HP:0012847	Epilepsia partialis continua	2	2	0.1	8.52
19	HP:0003688	Decreased activity of cytochrome C oxidase in muscle tissue	16	15	1	8.10
20	HP:0006915	Inability to walk by childhood/adolescence	1	1	0.1	7.52
21	HP:0007398	Asymmetric, linear skin defects	1	1	0.1	7.52
22	HP:0004871	Perineal fistula	1	1	0.1	7.52
23	HP:0004856	Normochromic microcytic anemia	1	1	0.1	7.52
24	HP:0003116	Abnormal echocardiogram	1	1	0.1	7.52
25	HP:0003115	Abnormal EKG	1	1	0.1	7.52
26	HP:0008335	Renal aminoaciduria	1	1	0.1	7.52
27	HP:0000650	Abnormal amplitude of pattern reversal visual evoked potentials	1	1	0.1	7.52
28	HP:0005941	Intermittent hyperpnea at rest	1	1	0.1	7.52
29	HP:0003329	Hair shafts flattened at irregular intervals and twisted through 180 degrees about their axes	1	1	0.1	7.52
30	HP:0005750	Contractures of the joints of the lower limbs	1	1	0.1	7.52
31	HP:0007620	Cutaneous leiomyoma	1	1	0.1	7.52
32	HP:0007190	Neuronal loss in the cerebral cortex	1	1	0.1	7.52
33	HP:0003434	Sensory ataxic neuropathy	1	1	0.1	7.52
34	HP:0012817	Noncompaction cardiomyopathy	1	1	0.1	7.52
35	HP:0100954	Open operculum	1	1	0.1	7.52
36	HP:0006755	Cutaneous leiomyosarcoma	1	1	0.1	7.52
37	HP:0008610	Infantile sensorineural hearing impairment	1	1	0.1	7.52
38	HP:0008587	Mild neurosensory hearing impairment	1	1	0.1	7.52
39	HP:0003209	Decreased pyruvate carboxylase activity	1	1	0.1	7.52
40	HP:0005201	Anomalous splenoportal venous system	1	1	0.1	7.52
41	HP:0008504	Moderate sensorineural hearing impairment	1	1	0.1	7.52
42	HP:0006961	Jerky head movements	1	1	0.1	7.52
43	HP:0006964	Cerebral cortical neurodegeneration	1	1	0.1	7.52
44	HP:0006558	Decreased mitochondrial complex III activity in liver tissue	1	1	0.1	7.52
45	HP:0006582	Reye syndrome-like episodes	1	1	0.1	7.52
46	HP:0003536	Decreased fumarate hydratase activity	1	1	0.1	7.52
47	HP:0009129	Upper limb amyotrophy	1	1	0.1	7.52
48	HP:0011318	Bicoronal synostosis	1	1	0.1	7.52
49	HP:0007713	Juvenile zonular cataracts	1	1	0.1	7.52
50	HP:0004901	Exercise-induced lactic acidemia	1	1	0.1	7.52
51	HP:0004900	Severe lactic acidosis	1	1	0.1	7.52
52	HP:0004902	Congenital lactic acidosis	1	1	0.1	7.52
53	HP:0005886	Aphalangy of the hands	1	1	0.1	7.52
54	HP:0040187	Neonatal sepsis	1	1	0.1	7.52
55	HP:0012712	Mild hearing impairment	1	1	0.1	7.52
56	HP:0008306	Abnormal iron deposition in mitochondria	1	1	0.1	7.52
57	HP:0012087	Abnormal mitochondrial shape	1	1	0.1	7.52
58	HP:0007437	Multiple cutaneous leiomyomas	1	1	0.1	7.52
59	HP:0005152	Oncocytic cardiomyopathy	1	1	0.1	7.52
60	HP:0005157	Concentric hypertrophic cardiomyopathy	1	1	0.1	7.52
61	HP:0003652	Recurrent myoglobinuria	1	1	0.1	7.52
62	HP:0012861	Ovotestis	1	1	0.1	7.52
63	HP:0005977	Hypochloremic metabolic alkalosis	1	1	0.1	7.52
64	HP:0007183	Focal T2 hyperintense basal ganglia lesion	1	1	0.1	7.52
65	HP:0006581	Depletion of mitochondrial DNA in liver	1	1	0.1	7.52
66	HP:0006467	Limited shoulder movement	1	1	0.1	7.52
67	HP:0009141	Depletion of mitochondrial DNA in muscle tissue	1	1	0.1	7.52
68	HP:0005198	Stiff interphalangeal joints	1	1	0.1	7.52
69	HP:0003232	Mitochondrial malic enzyme reduced	1	1	0.1	7.52
70	HP:0000576	Centrocecal scotoma	1	1	0.1	7.52
71	HP:0011925	Decreased activity of mitochondrial ATP synthase complex	1	1	0.1	7.52
72	HP:0030528	Paracentral scotoma	1	1	0.1	7.52
73	HP:0007344	Atrophy/Degeneration involving the spinal cord	1	1	0.1	7.52
74	HP:0006789	Mitochondrial encephalopathy	1	1	0.1	7.52
75	HP:0007146	Bilateral basal ganglia lesions	1	1	0.1	7.52
76	HP:0009062	Infantile axial hypotonia	1	1	0.1	7.52
77	HP:0003348	Hyperalaninemia	10	9	1	7.37
78	HP:0001404	Hepatocellular necrosis	18	16	2	7.20
79	HP:0002151	Increased serum lactate	83	73	10	7.06
80	HP:0008316	Abnormal mitochondria in muscle tissue	21	18	3	6.78
81	HP:0003128	Lactic acidosis	112	93	19	6.49
82	HP:0004481	Progressive macrocephaly	22	18	4	6.37
83	HP:0003548	Subsarcolemmal accumulations of abnormally shaped mitochondria	5	4	1	6.20
84	HP:0003542	Increased serum pyruvate	10	8	2	6.20
85	HP:0003737	Mitochondrial myopathy	5	4	1	6.20
86	HP:0003535	3-Methylglutaconic aciduria	9	7	2	6.00
87	HP:0001994	Renal Fanconi syndrome	12	9	3	5.78
88	HP:0012240	Increased intramyocellular lipid droplets	15	11	4	5.65
89	HP:0000590	Progressive external ophthalmoplegia	11	8	3	5.61
90	HP:0002875	Exertional dyspnea	14	10	4	5.52
91	HP:0003546	Exercise intolerance	61	42	19	5.34
92	HP:0003689	Multiple mitochondrial DNA deletions	6	4	2	5.20
93	HP:0006799	Basal ganglia cysts	3	2	1	5.20
94	HP:0000642	Red-green dyschromatopsia	3	2	1	5.20
95	HP:0006970	Periventricular leukomalacia	3	2	1	5.20
96	HP:0002181	Cerebral edema	29	19	10	5.12
97	HP:0003323	Progressive muscle w eakness	10	6	4	4.78
98	HP:0001085	Papilledema	10	6	4	4.78
99	HP:0011024	Abnormality of the gastrointestinal tract	5	3	2	4.78
100	HP:0001403	Macrovesicular hepatic steatosis	5	3	2	4.78
101	HP:0000124	Renal tubular dysfunction	15	9	6	4.78
102	HP:0002880	Respiratory difficulties	17	10	7	4.71
103	HP:0002878	Respiratory failure	61	35	26	4.62
104	HP:0003200	Ragged-red muscle fibers	14	8	6	4.61
105	HP:0002793	Abnormal pattern of respiration	27	15	12	4.52
106	HP:0002415	Leukodystrophy	40	21	19	4.34

Appendix IV. Distribution of phenotype scores for the three different query lists: the MitoAll gene set (blue), the MitoSDHrem gene set (red) and the SuperMito gene set (green).



Appendix V. Enriched biological processes and KEGG pathways among the top 200 non-mitochondrial genes (using the SuperMIto query).

KEGG pathways

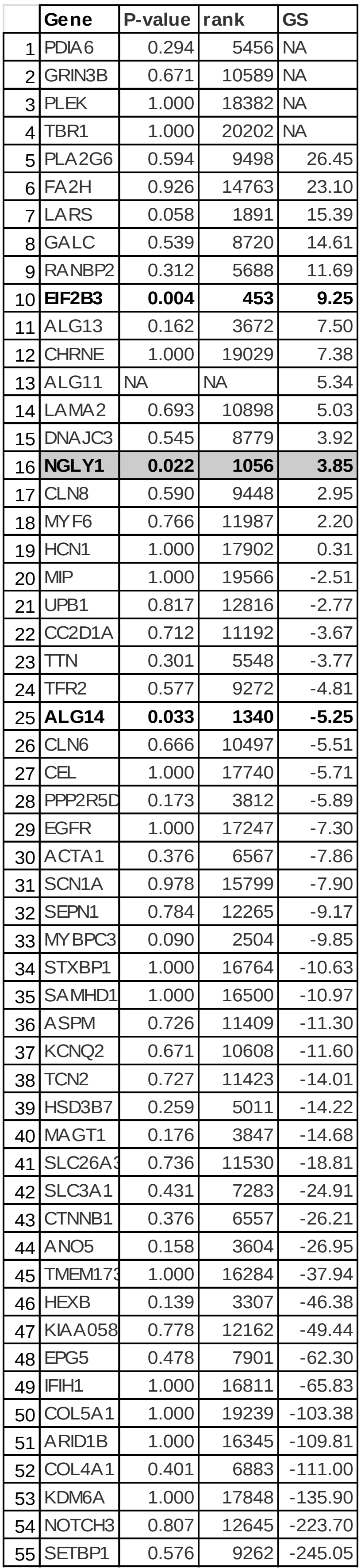
pathway ID	pathway description	No.	FDR	matching proteins
1100	Metabolic pathways	41	1.26e-10	ACAT2, ADK, ALDOB, ALG11, ALG6, ALG9, AMPD2, ASPA, ASS1, B4GALNT1, DDOST, DHFR, DPYD, DPYS, ENO3, FBP1, FTCD, GAD1, GALC, GAMT, GCH1, GLUL, KYNU, MAT1A, NDST1, NT5C2, PCK1, PLA2G6, PLCE1, PNPLA2, POLR3A, PPT1, RRM2B, SLC33A1, ST3GAL5, STT3A, STT3B, SYNJ1, TH, TPK1, TYMP
510	N-Glycan biosynthesis	7	5.59e-05	ALG11, ALG6, ALG9, DDOST, RFT1, STT3A, STT3B
4142	Lysosome	9	0.000257	ARSA, CTSF, GALC, LAMP2, MFSD8, NPC1, NPC2, PPT1, PSAP
4976	Bile secretion	7	0.000312	ADCY5, ATP1A3, SLC10A2, SLC2A1, SLC4A4, SLC5A1, SLC9A1
970	Aminoacyl-tRNA biosynthesis	5	0.00339	AARS, DARS, LARS, MARS, SEPSECS
250	Alanine, aspartate and glutamate metabolism	4	0.0155	ASPA, ASS1, GAD1, GLUL
604	Glycosphingolipid biosynthesis - ganglio series	3	0.0155	B4GALNT1, SLC33A1, ST3GAL5
240	Pyrimidine metabolism	6	0.0157	DPYD, DPYS, NT5C2, POLR3A, RRM2B, TYMP
4973	Carbohydrate digestion and absorption	4	0.0158	ATP1A3, PIK3R5, SLC2A2, SLC5A1
1230	Biosynthesis of amino acids	5	0.0172	ALDOB, ASS1, ENO3, GLUL, MAT1A
3013	RNA transport	7	0.0183	EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, NUP62, RANBP2
4964	Proximal tubule bicarbonate reclamation	3	0.0187	ATP1A3, PCK1, SLC4A4
4919	Thyroid hormone signaling pathway	6	0.0203	ATP1A3, MED17, PIK3R5, PLCE1, SLC2A1, SLC9A1
4141	Protein processing in endoplasmic reticulum	7	0.0218	BCAP31, DDOST, DNAJB2, SAR1B, STT3A, STT3B, UBQLN2
4911	Insulin secretion	5	0.0223	ADCY5, ATP1A3, SLC2A1, SLC2A2, SNAP25
4978	Mineral absorption	4	0.0235	ATP1A3, CLCN2, FTL, SLC5A1
10	Glycolysis / Gluconeogenesis	4	0.0442	ALDOB, ENO3, FBP1, PCK1
410	beta-Alanine metabolism	3	0.0442	DPYD, DPYS, GAD1

Biological processes

pathway ID	pathway description	No.	FDR	matching proteins
GO.0044281	small molecule metabolic process	58	4.59e-11	AARS, ABHD12, ADCY5, AIMP1, ALDOB, ARSA, ASPA, ASS1, CA8, CHAT, CHKB, DARS, DNM2, DPYD, ENO3, ERLIN2, FA2H, FBP1, FOLR1, FTCD, GAD1, GALC, GLUL, GNB4, LARS, MARS, MAT1A, MMAA, MTRR, NDST1, NPC1, NPC2, NT5C2, NUP62, PCK1, PHKA1, PIK3R5, PLA2G6, PLCE1, PLP1, PNKP, PNPLA2, PSAP, RANBP2, SAR1B, SLC10A2, SLC19A3, SLC2A1, SLC2A2, SLC5A1, SLC9A1, SNAP25, SYNJ1, TH, TPK1, TTR, TYMP, VAPB
GO.0044710	single-organism metabolic process	81	9.57e-10	AARS, ACAT2, ADCY5, AIMP1, ALDOB, ALG11, ALG6, ALG9, APTX, ARSA, ASPA, ASS1, CA8, CHAT, CHKB, CSF1R, CTSD, DARS, DDHD1, DDOST, DNAJB2, DNM2, DPYD, ENO3, ERLIN2, EXOSC3, FBP1, FBXO7, FOLR1, FTCD, GAD1, GALC, GLUL, GNB4, IGHMBP2, LARS, MARS, MAT1A, MMAA, MTRR, NPC1, NPC2, NT5C2, NUP62, PCK1, PCNA, PHKA1, PIK3R5, PLA2G6, PLCE1, PLP1, PNKP, PNPLA2, PPT1, PSAP, RANBP2, RFT1, SAR1B, SETX, SFTPB, SLC10A2, SLC19A3, SLC2A1, SLC2A2, SLC5A1, SLC5A7, SLC9A1, SNAP25, ST3GAL5, STT3A, STT3B, SYNJ1, TDP1, TH, TPK1, TYMP, UBQLN2, VAPB, VRK1, WDR45, ZFYVE26
GO.0008366	axon ensheathment	13	3.22e-09	ASPA, EGR2, EIF2B2, EIF2B4, EIF2B5, FA2H, JAM3, LAMA2, MTMR2, PMP22, PRX, SBF2, SH3TC2
GO.1901564	organonitrogen compound metabolic process	46	3.22e-09	AARS, ABHD12, ACY1, ADCY5, AIMP1, ALDOB, ARSA, ASPA, ASS1, ATP6AP2, CHAT, CHKB, DARS, DDOST, DPYD, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, ENO3, FOLR1, FTCD, GAD1, GALC, GLUL, IGHMBP2, LARS, MARS, MAT1A, MMAA, MTRR, NDST1, NT5C2, PLA2G6, PPT1, PSAP, SEPSECS, SFTPB, SLC19A3, SLC9A1, ST3GAL5, TH, TPK1, TYMP, VAPB
GO.0042552	myelination	12	4e-08	ASPA, EGR2, EIF2B2, EIF2B4, EIF2B5, FA2H, JAM3, LAMA2, MTMR2, PMP22, SBF2, SH3TC2
GO.1901566	organonitrogen compound biosynthetic process	33	4.77e-08	AARS, ADCY5, AIMP1, AMPD2, ASS1, B4GALNT1, CHAT, CHKB, DARS, DDOST, DHFR, DPYD, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, GAMT, GLUL, IGHMBP2, KYNU, LARS, MARS, MAT1A, MMAA, MTRR, NDST1, SEPSECS, ST3GAL5, TH, TPK1, TYMP, VAPB
GO.0044712	single-organism catabolic process	31	1.27e-07	ALDOB, ASL, ASPA, CTSD, DDHD1, DNAJB2, DPYD, DPYS, ENO3, ERLIN2, FBXO7, FTCD, GAD1, GALC, GLUL, LDHA, MMAA, MTMR2, MTRR, NT5C2, PHKA1, PLA2G6, PLCE1, PNPLA2, PPT1, SLC9A1, STT3B, SYNJ1, TYMP, UBQLN2, WDR45
GO.0006520	cellular amino acid metabolic process	19	4.08e-07	AARS, ABHD12, ACY1, AIMP1, ASPA, ASS1, DARS, DPYD, DPYS, FOLR1, FTCD, GAD1, GCH1, GLUL, LARS, MARS, MAT1A, MTRR, TH
GO.0043648	dicarboxylic acid metabolic process	11	5.17e-07	ASPA, ASS1, DHFR, FOLR1, FTCD, GAD1, GLUL, KYNU, MTRR, PCK1, TH
GO.0019752	carboxylic acid metabolic process	28	8.71e-07	AARS, ABHD12, ACY1, AIMP1, ALDOB, ASPA, ASS1, DARS, DPYD, DPYS, ENO3, FA2H, FOLR1, FTCD, GAD1, GCH1, GLUL, LARS, MARS, MAT1A, MMAA, MTRR, NPC1, PCK1, PLP1, SLC10A2, SLC2A1, TH
GO.0014003	oligodendrocyte development	8	1.17e-06	ASPA, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, FA2H, PLP1
GO.0043603	cellular amide metabolic process	24	1.59e-06	AARS, AIMP1, ASL, ASS1, ATP6AP2, B4GALNT1, DARS, DDOST, DHFR, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, FOLR1, FTCD, GALC, GCH1, IGHMBP2, LARS, MARS, MTRR, SEPSECS, ST3GAL5
GO.0021782	glial cell development	10	1.64e-06	ASPA, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, FA2H, LAMA2, PLP1, SH3TC2
GO.1901135	carbohydrate derivative metabolic process	31	1.7e-06	ABHD12, ADCY5, ALDOB, ALG11, ALG13, ALG6, ALG9, ARSA, DDOST, DPYD, DPYS, ENO3, FBP1, GALC, GAMT, LDHA, MAT1A, MTRR, NDST1, NPC1, NT5C2, PSAP, RFT1, RRM2B, SAR1B, SLC9A1, ST3GAL5, STT3A, STT3B, TH, TYMP
GO.0043436	oxoacid metabolic process	29	3.27e-06	AARS, ABHD12, ACY1, AIMP1, ALDOB, ASPA, ASS1, DARS, DPYD, DPYS, ENO3, FA2H, FOLR1, FTCD, GAD1, GCH1, GLUL, LARS, MARS, MAT1A, MMAA, MTRR, NDST1, NPC1, PCK1, PLP1, SLC10A2, SLC2A1, TH
GO.0044711	single-organism biosynthetic process	35	4.41e-06	ADCY5, ALDOB, AMPD2, ASS1, B4GALNT1, CHAT, CHKB, DHFR, DPYD, ENO3, FA2H, FBP1, GAMT, GCH1, GLUL, KYNU, MAT1A, MMAA, MTMR2, MTRR, PCK1, PCNA, PIK3R5, PLA2G6, PLCE1, PLP1, PNPLA2, RRM2B, SLC2A1, SLC5A7, ST3GAL5, SYNJ1, TH, TYMP, VAPB
GO.0005975	carbohydrate metabolic process	26	8.2e-06	AIMP1, ALDOB, ALG11, ALG6, ALG9, DDOST, ENO3, FBP1, GALC, LDHA, MTMR2, NPC1, NUP62, PCK1, PHKA1, RANBP2, RFT1, SAR1B, SLC2A1, SLC2A2, SLC5A1, SLC9A1, ST3GAL5, STT3A, STT3B, SYNJ1
GO.0043604	amide biosynthetic process	19	1.12e-05	AARS, AIMP1, ASL, ASS1, B4GALNT1, DARS, DDOST, DHFR, EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5, GCH1, IGHMBP2, LARS, MARS, SEPSECS, ST3GAL5
GO.0044723	single-organism carbohydrate metabolic process	22	1.72e-05	AIMP1, ALDOB, ALG11, ALG6, ALG9, B4GALNT1, DDOST, ENO3, FBP1, LDHA, MTMR2, NDST1, NPC1, PCK1, PHKA1, RFT1, SAR1B, SLC2A1, ST3GAL5, STT3A, STT3B, SYNJ1
GO.0044765	single-organism transport	54	1.72e-05	ABCA3, ADCY5, ATP1A3, BCAP31, CASQ1, CHAT, CHRNE, CLCN2, DDOST, DNAJC6, DNM2, EGR2, FBXO7, FOLR1, GAD1, GJB1, GJC2, GLUL, GRID2, GRIK2, KCNC1, KCNT1, KIAA0226, KIF1C, KIF5A, LAMP2, LRSAM1, MFSD8, NPC1, NPC2, PFN1, PLA2G6, PRSS12, PSAP, RAB7A, RANBP2, RFT1, SAR1B, SLC10A2, SLC19A3, SLC22A5, SLC33A1, SLC4A4, SLC52A1, SLC5A7, SLC9A1, SNAP25, SYNJ1, SYT2, TH, TTR, UCHL1, VAPB, VPS53

Appendix

Appendix VI: Genes that are included in the list of mutations found in multiple patients with secondary mitochondrial disorders. The WeGET results are shown in the second and third column: the p-value of the genes coexpressed with the SuperMito gene list and the corresponding rank in the total gene list that WeGET provides as output. The third colomn contains the gene scores compiled from the SuperMito query. Bold genes have significant co-expression (P<0.05).



	Gene	P-value	rank	GS
1	PDIA6	0.294	5456	NA
2	GRIN3B	0.671	10589	NA
3	PLEK	1.000	18382	NA
4	TBR1	1.000	20202	NA
5	PLA2G6	0.594	9498	26.45
6	FA2H	0.926	14763	23.10
7	LARS	0.058	1891	15.39
8	GALC	0.539	8720	14.61
9	RANBP2	0.312	5688	11.69
10	**EIF2B3**	**0.004**	**453**	**9.25**
11	ALG13	0.162	3672	7.50
12	CHRNE	1.000	19029	7.38
13	ALG11	NA	NA	5.34
14	LAMA2	0.693	10898	5.03
15	DNAJC3	0.545	8779	3.92
16	**NGLY1**	**0.022**	**1056**	**3.85**
17	CLN8	0.590	9448	2.95
18	MYF6	0.766	11987	2.20
19	HCN1	1.000	17902	0.31
20	MIP	1.000	19566	-2.51
21	UPB1	0.817	12816	-2.77
22	CC2D1A	0.712	11192	-3.67
23	TTN	0.301	5548	-3.77
24	TFR2	0.577	9272	-4.81
25	**ALG14**	**0.033**	**1340**	**-5.25**
26	CLN6	0.666	10497	-5.51
27	CEL	1.000	17740	-5.71
28	PPP2R5D	0.173	3812	-5.89
29	EGFR	1.000	17247	-7.30
30	ACTA1	0.376	6567	-7.86
31	SCN1A	0.978	15799	-7.90
32	SEPN1	0.784	12265	-9.17
33	MYBPC3	0.090	2504	-9.85
34	STXBP1	1.000	16764	-10.63
35	SAMHD1	1.000	16500	-10.97
36	ASPM	0.726	11409	-11.30
37	KCNQ2	0.671	10608	-11.60
38	TCN2	0.727	11423	-14.01
39	HSD3B7	0.259	5011	-14.22
40	MAGT1	0.176	3847	-14.68
41	SLC26A3	0.736	11530	-18.81
42	SLC3A1	0.431	7283	-24.91
43	CTNNB1	0.376	6557	-26.21
44	ANO5	0.158	3604	-26.95
45	TMEM17	1.000	16284	-37.94
46	HEXB	0.139	3307	-46.38
47	KIAA058	0.778	12162	-49.44
48	EPG5	0.478	7901	-62.30
49	IFIH1	1.000	16811	-65.83
50	COL5A1	1.000	19239	-103.38
51	ARID1B	1.000	16345	-109.81
52	COL4A1	0.401	6883	-111.00
53	KDM6A	1.000	17848	-135.90
54	NOTCH3	0.807	12645	-223.70
55	SETBP1	0.576	9262	-245.05